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Review

p53 and ribosome biogenesis stress: The essentials

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ABSTRACT

Cell proliferation and cell growth are two tightly linked processes, as the proliferation program cannot be executed without proper accumulation of cell mass, otherwise endangering the fate of the two daughter cells. It is therefore not surprising that ribosome biogenesis, a key element in cell growth, is regulated by many cell cycle regulators. This regulation is exerted transcriptionally and post-transcriptionally, in conjunction with numerous intrinsic and extrinsic signals. Those signals eventually converge at the nucleolus, the cellular compartment that is not only responsible for executing the ribosome biogenesis program, but also serves as a regulatory hub, responsible for integrating and transmitting multiple stress signals to the omnipotent cell fate gatekeeper, p53. In this review we discuss when, how and why p53 is activated upon ribosomal biogenesis stress, and how perturbation of this critical regulatory interplay may impact human disease.

1. Introduction

Transformation of normal cells into cancer cells requires dysregulated activity of oncogenes that drive cellular proliferation and survival, alter metabolism and promote invasion into adjacent tissue. Additionally, neoplastic transformation requires inactivation, through mutations, deletions or epigenetic silencing, of tumor suppressor genes that monitor cell homeostasis, block unscheduled proliferation and prevent illegitimate cell survival.

When considering cellular pathways regulated by oncogenes and tumor suppressors, it is not immediately obvious that ribosome biogenesis should be among those pathways. However, a significant body of evidence accumulated over the last 10–15 years suggests that alterations of one or more steps that control ribosome biogenesis are essential for malignant transformation and progression, as many key tumor suppressors and proto-oncogenes have been found to regulate this process (Table 1). Among them, c-MYC and the components of the PI3K-mTORC1 signaling pathway are emerging as key regulators of ribosome biogenesis. So why exactly is a seemingly innocuous process found in the midst of a battleground between powerful positive and negative cellular regulators? The basic explanation is actually rather simple. Cancer is characterized by uncontrolled proliferation of cells, occurring relatively independently of external stimuli [1]. Yet, cell proliferation cannot take place without proper cell growth, namely an increase in cell mass. The increment in cell mass requires extensive protein synthesis, which is dependent on a constant supply of new ribosomes, effectively coupling ribosome biogenesis and protein synthesis to the cell cycle [2]. This is presumably the reason why genes like c-MYC, which control cell cycle progression and DNA synthesis, have evolved to also coordinate ribosome biogenesis and protein biosynthesis [3]. While c-MYC evolved to promote cell proliferation and growth, both under normal conditions and as a driver of malignancy, tumor suppressors like p53 and ARF, one of the two products of the *INK4a* locus, co-evolved as inspectors of cell homeostasis and emerged as gatekeepers of both genomic integrity and ribosome biogenesis.

Reflected by the large number of factors that regulate ribosome biogenesis, the construction of new ribosomes is an elaborate, well-coordinated process, and extremely demanding in terms of energy and resources [4]. It requires the activity of all three RNA polymerases, in order to transcribe both the rRNA and the mRNAs encoding about 80 distinct integral ribosomal proteins (RPs) and other accessory proteins. Ribosome biogenesis also impinges heavily upon the translation apparatus [4] and the nuclear import/export machinery [5,6].

Within the cell, the nucleolus is the main site of ribosome biogenesis (Fig. 1A). It is a sub-nuclear compartment where clusters of

Abbreviations: RPs, ribosomal proteins; NOR, nucleolar organizing regions; ActD, Actinomycin D; MDS, myelodysplastic syndrome; DBA, Diamond Blackfan anemia; SDS, Schwachman–Diamond syndrome; PTM, post translational modifications; 5′TOP, 5′ terminal oligopyrimidine

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Table 1
A list of reported participants in ribosome biogenesis stress signaling (upper) and key regulators of ribosome biogenesis (lower).

Activators of p53 following ribosome biogenesis stress	
Ribosomal proteins	RPL5 [65], RPL11 [64], RPL23 [66,128], RPS7 [129], RPL26 [91,92], RPS14 [96], RPS3 [130], RPL37 [131], RPS15 [131], RPS20 [131], RPS26 [132], RPS27 [133], RPS27L [133], RPS25 [134]
RNA	5S rRNA [84–86]
Accessory factors	PICT1 [88], nucleostemin [135], SRSF1 [89] NPM [136], NCL [93,137]
Regulators of ribosome biogenesis	
Oncogenic pathways	c-MYC (reviewed in [3]), E2F [138], AKT [139], mTOR (reviewed in [140]), ERK (reviewed in [11])
Tumor suppressors	p53 (reviewed in [10,11]), ARF (reviewed in [115]), PTEN [141], pRB (reviewed in [11])

tandem repeats of rRNA genes are organized into what is known as nucleolar organizing regions (NOR). The rRNA genes are transcribed by RNA polymerase I (PolI) to produce the precursor 47S rRNA with concurrent processing into mature rRNA species [7], followed by assembly of the rRNA together with RPs to form the 40S and 60S ribosomal subunits (Fig. 1A). Notably, different steps of the ribosome biogenesis process are misregulated in a variety of human malignancies, including cancer. A growing number of reports uncover a more complicated picture, where altered activity of the ribosome biogenesis machinery is not merely required to support the rapid proliferation of neoplastic cells, but it might also serve as a driving force in malignancy [8,9]. Thus, the activity of RNA PolI is dysregulated in cancer and other human pathologies [10,11], and enhanced rRNA transcription might attenuate the activity of tumor suppressor genes [8]. In addition to excessive RNA PolI activity, many RPs are overexpressed in human tumors such as colorectal cancer [12,13], esophagus cancer [14] and hepatocellular carcinoma [15]. Cancer cells might benefit from the dysregulation of specific RPs expression, as this might alter quality or quantity of the synthesized tumor promoting proteins [9] or even provide some non-ribosomal advantageous features [16].

Remarkably, alongside its role as the hub of ribosome biogenesis, the nucleolus also evolved into a highly sensitive regulatory hub, which is able to sense various stress signals and initiate a plethora of signaling cascades [17]. Of particular interest is a newly recognized signaling pathway involving ribosomal proteins RPL11 and RPL5 as well as 5S rRNA, which has a unique role in conveying stress messages upon impairment of ribosome biogenesis directly to the Mdm2/p53 module [18]. In this review we describe how the nucleolus and the ribosome biogenesis apparatus serve as unique transmitters of multiple stress signals, which impinge on the tumor suppressor transcription factor p53. We summarize the current knowledge regarding the mechanisms of p53 activation following ribosomal stress, and discuss how malfunctions in the ribosome biogenesis machinery can promote tumorigenesis and how this knowledge might be harnessed towards improving cancer therapy.

2. The nucleolus as a stress sensor

Because the process of ribosome biogenesis is extremely demanding in terms of energy and resources, its fidelity is closely inspected and virtually any type of severe cellular stress will result in an immediate shutdown of rRNA transcription (Fig. 1B) [19]. In response to such stress conditions, including exposure to different genotoxic agents like doxorubicin or inhibition of rRNA transcription using low levels of Actinomycin D (ActD), the nucleolus undergoes distinct structural changes, including condensation and segregation into structures called nucleolar caps, composed of nucleolar proteins and RNA [17,20,21]. Consequently, detection

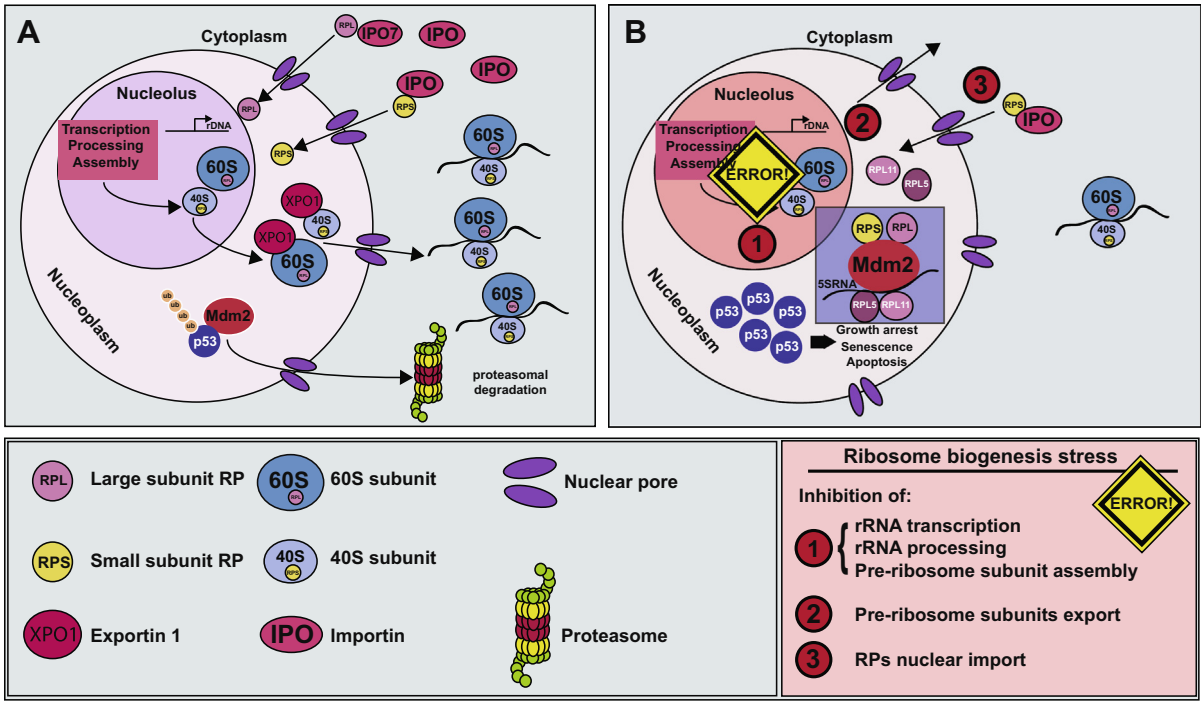

Fig. 1. An overview of ribosome biogenesis, both under normal and stress conditions. (A) Under basal conditions, rRNA is transcribed and processed in the nucleolus. RPs are imported into the nucleolus, where they are assembled together with processed rRNA into the large and small ribosomal subunits (60S and 40S, respectively). Mdm2 binds p53 and polyubiquitylates it, sending it to proteasomal degradation, possibly assisted by nucleolar-mediated export. (B) Inhibition of different steps in ribosome biogenesis can cause unassembled RPs and 5S rRNA to bind Mdm2 and prevent p53 degradation.

of such structural alterations might be considered as indicative of severe nucleolar stress. In other cases of milder stress, for example when ribosome biogenesis is suppressed by depletion of Rps6, nucleolar morphology is not distinctly altered [22]. Of note, many types of cellular stress, including Hypoxia [23], heat shock [23] and growth factor deprivation [24], were reported to activate p53 by blocking different steps of ribosome biogenesis and inducing nucleolar stress (Fig. 1B).

DNA damage has long been known to activate p53 through a variety of mechanisms [25,26], yet a revolutionary concept was proposed by Rubbi and Milner [23]. In a vintage experiment they showed that directed DNA damage, using localized UV irradiation, failed to activate p53 when the nucleolus remained unaffected, leading them to conclude that DNA damage alone cannot activate p53 and that nucleolar disruption is a prerequisite for p53 activation. However, future research will be necessary to uncover the underlying mechanisms by which DNA damage inhibits various steps of ribosome biogenesis and understand how various signaling pathways triggered by this type of stress are coordinated during p53 activation.

The first demonstration of a direct connection between p53-dependent cell cycle arrest and inhibition of ribosome biogenesis was provided by Pestov and colleagues [27], who showed that expression of a dominant negative form of Bop1, an rRNA maturation factor, blocks rRNA processing and activates p53-dependent growth arrest. Additional experimental models were subsequently employed to examine the activation of p53 following attenuation of ribosome biogenesis at multiple stages, such as by inhibition of rRNA transcription following the knockout of TIF-1A [28] or the transient knockdown of RNA PolI catalytic subunit *POLR1A* [29]. A p53 response can also be elicited by blocking rRNA processing or ribosome subunit assembly, through the stable or transient knockdown of many RPs or ribosomal accessory proteins [18]. Furthermore, ribosomal biogenesis is regulated not only at the stages of biosynthesis and assembly of its various components. Ribosomal proteins are synthesized in the cytoplasm, imported into the nucleus for assembly in the nucleolus, and then exported back into the cytoplasm as mature ribosomal subunits (Fig. 1A). This places substantial demands on the nuclear import and export machineries, particularly in light of the large amounts of ribosome-relevant cargo. Perturbation of nuclear import/export can therefore also elicit ribosomal biogenesis stress. Indeed, creating imbalance in the nuclear import of RPs through the knockdown of a single nuclear import factor, Importin 7 (IPO7), suffices to trigger p53 activation; this activation is dependent on RPL5 and RPL11, confirming that it emerges through *bona fide* ribosome biogenesis stress (Fig. 1B) [30]. The molecular mechanisms by which these perturbations of ribosome biogenesis trigger the p53 response will be discussed below.

3. Activation of p53 upon impairment of ribosome biogenesis *in vivo*

What are the in vivo manifestations of ribosomal stress-dependent activation of p53? As the first example, deletion of one mouse Rps6 allele in the T cell lineage prevented proliferation of those cells following receptor stimulation and reduced their accumulation in the spleen and lymph nodes [31], an effect that was mediated by p53. Furthermore, whole body deletion of a single *Rps6* allele resulted in embryonic lethality during gastrulation, apparently due to a p53-dependent checkpoint being triggered, as crossbreeding with a p53-null mouse strain bypassed their lethality and allowed their development until mid-gestation, when they most likely died as a result of defective translation of specific mRNAs [32]. More recently, the role of p53 in a number of mouse models for RP deficiencies has been demonstrated, both in preventing and mediating specific pathological manifestations [18].

Impairment of ribosome biogenesis is emerging as an important cause of human diseases. Mutations and deletions of several genes encoding RPs and ribosome biogenesis factors were found to underlie a group of congenital disorders known as ribosomopathies [33]. Diamond-Blackfan anemia (DBA) is the paradigm for this type of disorders. DBA is characterized by hypoplastic macrocytic anemia and specific developmental defects [34]. DBA patients are predisposed to the development of myelodysplastic syndrome (MDS) as well as acute myeloid leukemia and solid malignancies [35]. The most common genetic alterations in DBA are found within the RPS19 gene, but mutations and deletions were also found within several other RP genes [33,36]. Hapoloinsufficiencies of *RPS14* and *RPSA* were also identified in the 5q- syndrome, an acquired somatic deletion of chromosome 5q [37], and isolated congenital asplenia [38], respectively. Mutations in several other ribosome biogenesis factors have been identified as causes of ribosomopathies, including the Schwachman–Diamond syndrome (SDS), Treacher-Collins syndrome, dyskeratosis congenita and cartilage hair hypoplasia [39]. Recent studies of animal models of ribosomopathies and patients with ribosomopathies support a role for p53 in mediating specific pathological manifestations of these disorders [18]. Both zebrafish and mouse models of DBA revealed a role for p53 in the erythroid deficiency, and p53 deletion was found to rescue the erythroid phenotype in both these animal models [40,41]. Recent evidence suggests that activation of p53 underlies the pathogenesis of the human DBA, 5q- syndrome and SDS, as accumulation of nuclear p53 was found in bone marrow biopsy samples from these patients. Knockdown of RPS19 or RPS14 in human hematopoietic progenitor cells, mimicking common alterations in DBA and 5q- syndrome, respectively, was found to selectively activate p53 in the erythroid lineage, probably due to a lower threshold required for p53 activation in this lineage [42]. Importantly, lenalidomide, a common drug used to treat both DBA and 5q- syndrome, was recently shown to exert its therapeutic power through the inhibition of p53 [43]. However, the molecular mechanisms whereby p53 mediates the ribosomal biogenesis stress checkpoint pathway in ribosomopathies remain to be elucidated.

4. Mechanisms of p53 activation following ribosome biogenesis stress

How is p53 activated following ribosome biogenesis stress? In order to address this question, it is necessary to briefly discuss the mechanisms of p53 regulation by other types of stress signals. In unstressed cells, p53 levels are typically very low. These low levels are maintained in great part through the activity of p53's E3 ubiquitin ligase Mdm2 [44]. Mdm2 and p53 form a negative feedback loop, in which p53 positively regulates the transcription of the *Mdm2* gene, while Mdm2 in turn ubiquitylates p53 and thereby promotes its proteasome-mediated degradation [45–47]. This activity was proposed to require the shuttling of the p53/Mdm2 complex into the cytoplasm, where both proteins are degraded by the proteasome, although subsequent reports indicate that proteasome-mediated p53 degradation can also take place in the nucleus [48,49], and even in the nucleolus in a ubiquitin-independent, calpain-dependent manner [50]. Additionally, Mdm2 binds the N-terminus of p53, blocking its transcriptional activity. In order to activate p53, this loop must be interrupted. One way for this to happen is through post-translational modifications (PTMs) such as phosphorylation [51,52] and acetylation [53,54] of p53 as well as Mdm2 [55,56]. These modifications impair p53/Mdm2 interaction and enhance p53 tetramerization and its binding to p53 responsive

elements within the DNA. The result of p53 activation will usually depend on the context and severity of the imposed stress. For example, while mild DNA damage might trigger p53-dependent growth arrest and promote DNA repair, severe damage will often result in either p53-dependent cellular senescence or apoptosis.

PTMs of p53 are not the only route to p53 activation. Another way to disengage p53 from the deadly grip of Mdm2 is through binding of modulator proteins to Mdm2, in a way that either directly prevents Mdm2-p53 binding or sequesters Mdm2 in a different cellular compartment [57]. The best example is provided by ARF. This potent tumor suppressor protein accumulates upon excessive mitogenic signaling such as c-MYC overexpression, and induces p53-dependent apoptosis or growth arrest [58]. Mechanistically, ARF activates p53 by blocking Mdm2 function through its ability to bind the acidic domain of Mdm2 [59,60] and hinder its E3 ubiquitin ligase activity.

In a similar fashion to ARF, a number of RPs were found to bind Mdm2 and activate p53. The first indication that ribosomal elements can communicate directly with the p53 pathway was provided more than 20 years ago, when the 5.8S rRNA was reported to be covalently linked to p53 [61,62]. This was followed by the demonstration that the L5/5S rRNA complex can be co-immunoprecipitated with Mdm2 and p53 [63]. The functional impact of this interaction was unknown at the time, and it was speculated that the L5/5S rRNA/p53-Mdm2 complex might participate in ribosome biogenesis control or alternatively may selectively block the translation of specific, cell cycle-related mRNAs [63].

The fact that RPs can regulate p53 activity in a similar fashion to ARF was first demonstrated for RPL5, RPL11 and RPL23 [64–66]. Since then, many other RPs were reported to bind to Mdm2 and elicit a p53 response in a similar fashion (Fig. 2A and Table 1). Overexpression of RPL11, RPL5 and RPL23 was found to impede Mdm2 ubiquitin ligase activity and stabilize p53. Additionally, treatment of cells with low levels of ActD, specifically inhibiting ribosome biogenesis, was shown to increase the binding of RPL5 and RPL11 to Mdm2. Somewhat similar to ARF, the binding of the different RPs was mapped to the central domain of Mdm2, yet to a slightly different region than ARF [67]. Specifically, it was demonstrated that a cancer-associated mutation causing a cysteine to phenylalanine substitution at position 305 within the Mdm2 zinc finger domain ($Mdm2^{C305F}$), located downstream to the central acidic domain, is sufficient to abort the interaction of Mdm2 with L5 and L11 and the subsequent p53 activation [68].

As mentioned above, many oncogenes and oncogenic pathways that promote ribosome biogenesis (Table 1) can also trigger a potent p53 response [58,69,70]. Among these, c-MYC and RAS are probably the most conspicuous. RAS is now believed to exert its effect on p53 primarily through DNA replication stress and a DNA damage response [71,72], although additional mediators such as the tumor suppressors ARF [58,73] and Lats2 also contribute to RAS-induced p53 activation [74]. In contrast, ARF was suggested to be the main mediator of p53 activation following c-MYC overexpression [58]. However, this inference is challenged by the analysis of mice expressing the $Mdm2^{C305F}$ mutant, crossbred with Eμ-myc mice. Notably, development of lymphoma in this c-MYC overexpression model was accelerated independently of ARF [75], suggesting that excessive production of RPL5 and RPL11 when c-MYC is hyperactive triggers the RPL5/RPL11/Mdm2/p53 ribosomal stress checkpoint signaling pathway to prevent malignant transformation and tumor progression. Using the same $Mdm2^{C305F}$ mouse model, it was subsequently found that in contrast to c-MYC deregulation, the p53 response was not hampered when RAS was overexpressed [76]. This is particularly intriguing in light of the dogma that ARF is the main activator of p53 in the face of deregulated c-MYC, but might be less important for RAS-dependent p53 activation in the human context [77].

The list of the RPs whose deregulation may promote p53 activation is still growing, but two of those RPs, RPL5 and RPL11, seem to have a unique and exclusive role in p53 regulation following ribosome biogenesis stress. It is important to note that knockdown of either RPL11 or RPL5 separately results in abrogation of an adequete p53 response in face of several ribosome biogenesis stress signals. Interestingly, knockdown of many other RPs, including some of those described as activators of p53, can by itself disrupt ribosome biogenesis and elicit a p53 response, yet this is not the case for RPL11 and RPL5 [78,79]. Although partial depletion of either RPL5 or RPL11 results in growth arrest, this is rather due to a global reduction in protein synthesis and not because a p53 activation program has been executed [78]. The critical role of both RPL5 and RPL11 in p53 activation following ribosome biogenesis impairment accentuates the observation that, upon exposure of cells to various ribosome biogenesis stressors, endogenous RPL5 and RPL11 are unique among several other RPs examined in being able to accumulate in the non-ribosomal fraction, where they specifically bind Mdm2 [80]. Furthermore, the significance of RPL5 and RPL11 in p53 activation upon inhibition of ribosome biogenesis or upon c-MYC overexpression is convincingly supported by the analysis of the $Mdm2^{C305F}$ mouse model [75].

How exactly do the different RPs mediate the p53 stress response? Following translation, RPs are shuttled from the

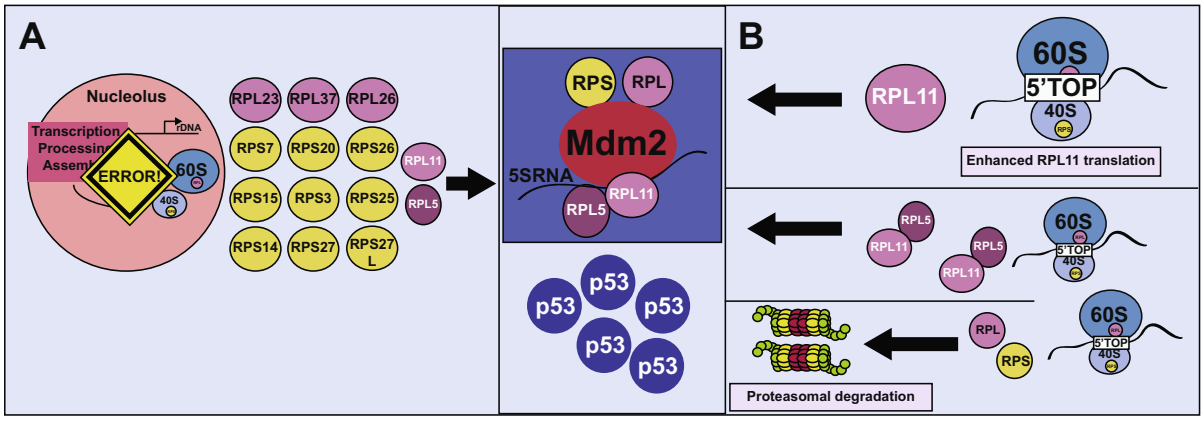

Fig. 2. Suggested models of p53 activation by ribosomal biogenesis stress. (A) The RPs diffusion model: following a stress signal, RPs are free to diffuse from the nucleoplasm, where they bind Mdm2. The different RPs that were reported to bind Mdm2 are depicted (see also Table 1). (B) Translational source of p53 activating RPs. Upper panel: Following depletion of the 40S subunit, RPL11 mRNA translation in a 5′TOP-dependent manner is enhanced, causing RPL11 to accumulate and activate p53. Lower panel: Subsequent to ribosome biogenesis stress, newly synthesized RPL5 and RPL11 are imported into the nucleus, where they bind Mdm2 and activate p53. Other RPs fail to enter the nucleus and are quickly degraded.

cytoplasm into the nucleus in an energy-dependent manner, assisted by the beta-karyopherin family members [81,82]. RPs are basic proteins, which aggregate quickly in the cytoplasm if not bound and transported by the import machinery [83]. After nuclear import, RPs accumulate in the nucleolus, where they are assembled together with rRNA into ribosomal subunits (Fig. 1A). There has been considerable controversy regarding the source of p53-activating RPs upon ribosome biogenesis stress. It was initially perceived that perturbed ribosome biogenesis, resulting in nucleolar disruption, impel the diffusion of a number of RPs from the nucleolus into the nucleoplasm, where they are free to bind Mdm2 and activate p53 (Fig. 2A) [67]. Yet, new evidence is now challenging this dogma, raising alternative assumptions regarding the source and type of the RPs that signal to p53 activation [18,80].

Although some impairments of ribosome biogenesis do not cause an obvious nucleolar damage, they still can activate p53 in an RPL11/Mdm2-dependent manner. For example, knockdown of RPS6 interrupts the biogenesis of the 40S small ribosomal subunit and activates p53, yet without evident disruption of nucleolar integrity [22]. In that case, mRNAs containing 5′TOP are preferentially translated, including that of *RPL11* and possibly other RPs, resulting in accumulation of excessive free RPL11 protein and subsequent p53 activation (Fig. 2B) [22].

The most recent model also implicates protein synthesis as the source of p53-activating RPs upon impairment of ribosome biogenesis. It proposes that under such conditions all RPs are normally synthesized (Fig. 2B) [80]. However, while other RPs are quickly dismissed via proteasome-mediated degradation, RPL5 and RPL11 are protected from degradation and continue to be imported into the nucleolus where they bind Mdm2 and activate p53 (Fig. 2B) [80], implying that RPL11 and RPL5, each independently yet mutually, are essential factors in mediating the ribosome biogenesis stress signal to p53. We have recently demonstrated that depletion of a single nuclear import factor, Importin 7 (IPO7), triggers a p53 response that is dependent on both RPL5 and RPL11 [30]. These two RPs are likely imported via several parallel importins [5,81], and are thus not dependent on a single import factor. Hence, while the import of other RPs such as RPL4 is impaired upon IPO7 depletion, leading to ribosome biogenesis stress, sustained import of RPL5 and RPL11 by other import factors allows p53 activation under these conditions (Fig. 1B).

In addition to RPL5 and RPL11, recent reports highlight also the importance of 5S rRNA, as part of the 5S RNP complex, as a necessary element in ribosome biogenesis stress signaling to p53. Specifically, depleting 5S rRNA with the aid of siRNA targeted against the PolIII transcription factor TIFIIIA prevents ribosomal biogenesis stress-dependent activation of p53 [84,85]. In addition, siRNA targeting 5S rRNA directly was found to lower the levels of Mdmx, an Mdm2-related protein required for maximizing the E3 ligase activity of Mdm2, thereby enhancing p53 activity [86]. Thus, it seems that under conditions of ribosome biogenesis stress, newly synthesized RPL5/RPL11/5S rRNA complex is redirected from ribosome biogenesis to the Mdm2/p53 module (Fig. 2B).

Signaling through the RPL11/RPL5/5S complex is also supported by additional factors such as RPL37, which is degraded following DNA damage, inducing RPL11 binding to Mdm2 [87]. Non-ribosomal proteins also assist with this process, as exemplified by the nucleolar protein PICT1, which regulates RPL11 release from the nucleolus upon ribosomal stress [88], and the splicing factor SRSF1, which cooperates with RPL5 in p53 activation and driving of normal fibroblasts into senescence [89].

RPs can also convey the stress message to p53 via alternative mechanisms. Of note, RPL11 was found to enhance p53 transcriptional activity through its ability to bind p53 target genes and augment recruitment of the p300/CBP acetyltransferases and p53 acetylation [90]. These authors also reported that the Neddylation of RPL11 is crucial for its ability to alleviate Mdm2-dependent repression of p53 transcriptional activity. Another unique example is provided by RPL26: although, like many other RPs, RPL26 can bind Mdm2 and activate p53 in the canonical manner [91], this RP also binds and enhances the translation of p53 mRNA [92,93]. Under basal conditions RPL26 is bound to Mdm2, which prevents it from enhancing p53 mRNA translation. Furthermore, RPL26 is a direct target for Mdm2-mediated polyubiquitylation and degradation. However, upon genotoxic stress the inhibitory effect of Mdm2 over RPL26 is attenuated, enabling rapid p53 translation [92]. Strikingly, TGF-β1 can dampen the cellular stress response by hindering the ability of RPL26 to enhance p53 mRNA translation [94].

Conversely, some RPs can mediate tumor suppression independently of p53. For instance, both RPL11 and RPS14 were reported to bind the N-terminal MBII domain of c-MYC and avert the binding of the c-MYC coactivator TRRAP [95–97], thereby inhibiting c-MYC-mediated transactivation of target genes. Additionally, RPL11 and RPL5 bind the 3′UTR of *c-MYC* mRNA, leading to its microRNA/RISC-mediated degradation [98,99]. Moreover, RPS3 was found to have a dual activity of both enhancing DNA damage repair, thereby contributing to genome stability [100], and mediating apoptosis [101,102].

5. The p53-ARF axis constrains ribosome biogenesis

In light of the oncogenic potential of the protein synthesis apparatus, it is perhaps not surprising that in addition to being activated in response to deregulated ribosome biogenesis, p53 can also curb ribosome biogenesis, either through its activity as a transcription factor or through direct binding to different elements in this pathway (Fig. 3). Thus, p53 can quench the activity of RNA PolI and RNA PolIII, in the latter case through direct binding to TFIIIB [103,104]. In the case of PolI, p53 was shown to hinder the assembly of the RNA PolI initiation complex via its interaction with SL1, with resultant attenuation of the formation of the SL1/UBF complex [105]. In addition, p53 constrains rRNA transcription indirectly through several mechanisms. For example the CDK inhibitor p21, product of one of p53's main transcriptional target genes, activates the retinoblastoma tumor suppressor protein (pRB), a potent inhibitor of rRNA transcription [106,107]. Additionally, p53 can impede ribosome biogenesis indirectly by

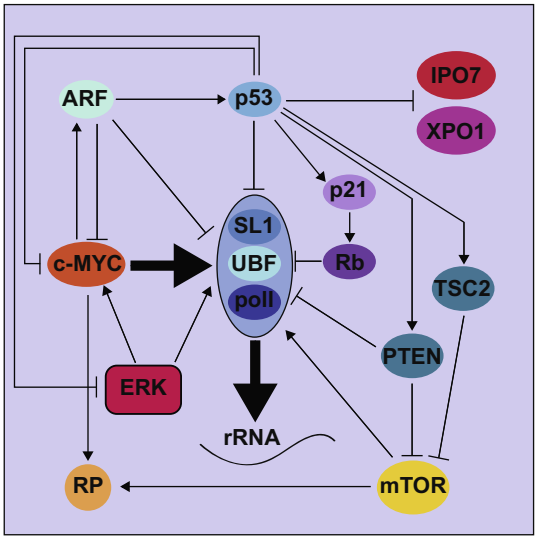

Fig. 3. p53 inhibits ribosome biogenesis via direct and indirect mechanisms. See text for further details.

suppressing the expression and activity of c-MYC [108,109] and mTOR [110,111], attenuating ribosome biogenesis at multiple levels (Fig. 3).

One intriguing possibility is that p53 may influence the trafficking of RPs and ribosomal subunits. Induction of DNA damage alters the subcellular accumulation of many RPs in p53-proficient but not p53-deficient cells [112].Strikingly, p53 represses the transcription of the *IPO7* gene as well as that of *Exportin 1* (XPO1) [30,113], better known as CRM1 – the main protein responsible for the nuclear export of assembled ribosome subunits [114]. Remarkably, the same genes are upregulated by c-MYC. Thus activated p53 may dampen the nuclear traffic of ribosomal proteins and ribosomal subunits, stalling ribosome biogenesis in response to genotoxic stress and coordinating cell cycle arrest with the attenuation of protein synthesis. As p53 can also influence the overall levels of RPs via multiple direct and indirect mechanisms (Fig. 3), further experimental evidence will be required to establish a direct role for p53 in regulating the shuttling of RPs and ribosome subunits.

As discussed above, ARF is a major component of the p53 pathway. Remarkably, ARF also possesses a p53-independent ability to constrain ribosome biogenesis [115] (Fig. 3). ARF can obstruct rRNA processing [116,117], as well as interfere with rRNA transcription through direct binding to rDNA gene promoter regions and via interference with UBF phosphorylation [118,119]. Additionally, ARF can affect rRNA transcription via sequestration of TTF-I outside of the nucleolus [120] and inhibition of Mdm2-dependent ubiquitination of TTF-I [121]. Consistent with these findings, recent reports suggest that ARF can trigger the RPL5/RPL11/Mdm2/p53 checkpoint via suppression of several steps of ribosome biogenesis and physically and functionally interacting with the p53-activating RPL11 under these conditions [85,122]. Together, these findings underscore the complexity of ribosome biogenesis regulation by the p53-ARF axis, suggesting that this tumor suppressive pathway constitutes an important barrier against tumorigenesis.

6. The ribosome biogenesis machinery as a putative cancer therapy target

The understanding that excessive ribosome biogenesis promotes tumorigenesis but this machinery also serves as a fundamental component of the stress signaling apparatus, led several groups to try and develop anti-cancer treatments exploiting these two features. Although in about 50% of all human cancers p53 is inactivated via mutations or deletions, the remainder half retain wild type (WT) p53, apparently incapacitated as a tumor suppressor. As discussed above, p53 activation can be triggered by suppression of ribosome biogenesis. This notion has led to the idea of treating tumors harboring WT p53 with a low dose of ActD, thereby inhibiting RNA PolI activity while minimizing non-specific adverse effects [123]. Other chemotherapeutic agents like 5-FU, cisplatin, temsirolimus, mitomycin C and irinotecan/topotecan, were also shown to exert some of their anti-cancer effects via obstructing RNA PolI activity [10]; however, all of them also induce genomic damage and other adverse effects. Recently, high specificity RNA PolI inhibitors were developed: the small molecule CX-5461 specifically blocks the binding of SL1 to the promoter of rDNA genes, thus preventing rRNA transcription [124]. Strikingly, while CX-5461 induces a very potent p53 response in blood malignancies [125], it can also restrain the growth of human solid tumors independently of p53 [124]. Another small molecule, BMH-21, was recently reported to target PolI activity through its ability to promote the proteasomal degradation of RPA194, the large catalytic subunit of PolI holocomplex [126]. Although BMH-21 intercalates into DNA with high affinity to GC-rich regions, it does not elicit a DNA damage response [127] and its ability to activate p53 is probably mediated by ribosome biogenesis stress. This promising agent was shown to effectively impede the growth of different cancer cells, both in vitro and in vivo [126]. Both cases serve as a proof of concept, demonstrating how specific targeting of RNA PolI can strike tumors at two key points in parallel, suppressing a core cellular function required for the fast proliferation of cancer cells while activating an anti-tumoral p53 response.

7. Conclusions

Our growing understanding of the biology of ribosome biogenesis unexpectedly opened up a new and exciting field in cancer biology. As evidence accumulates, we can now appreciate the role of p53 in both sensing the fidelity of ribosome biogenesis as well as constraining it, thereby coordinating cell growth with cell cycle progression and mediating an additional layer of protection against cancer.

Although it has been known for many years that the ribosome biogenesis machinery is dysregulated in cancer, we now understand that inherited and acquired abnormalities in ribosome function can lead to tumorigenesis and thus it can be speculated that activation of a p53-dependent checkpoint response might prevent expansion of such potentially hazardous cells. Although there are some indications in support of this idea, definitive evidence has not yet been provided. As patients of DBA and other ribosomopathies are predisposed to various malignancies, it is imperative to understand whether chronic p53 activation in ribosomopathy-derived cells is a driving pressure for p53 inactivation by mutations, deletions or epigenetic mechanisms, enabling neoplastic growth. Additionally, p53-downstream signaling pathways that mediate its effect of other pathological phenotypes in ribosomopathies need to be uncovered.

Many open questions still linger regarding the mechanisms of p53 activation following ribosomal stress. The spatial and temporal interaction of the p53/Mdm2 module with RPs is still largely unresolved, including the ability of the nucleolus to regulate p53 localization, shuttling and degradation. Moreover, we do not fully understand why RPL5 and RPL11 are so unique in activating p53, when so many other RPs are capable of binding Mdm2.

Transferring the current knowledge in this field from mostly in vitro models to in vivo mouse models will constitute the next phase in utilizing the p53-RP connection for better understanding of cancer biology, hopefully harnessing this knowledge towards the introduction of new and improved cancer treatments.

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